

13/3,AB/9 (Item 1 from file: 370)

DIALOG(R) File 370: Science

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00510315

Replication of Subgenomic Hepatitis C Virus RNAs in a Hepatoma Cell Line

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Language: English

Section Heading: REPORTS

Word Count: 2866

Abstract: An estimated 170 million persons worldwide are infected with hepatitis C virus (HCV), a major cause of chronic liver disease. Despite increasing knowledge of genome structure and individual viral proteins, studies on virus replication and pathogenesis have been hampered by the lack of reliable and efficient cell culture systems. A full-length consensus genome was cloned from viral RNA isolated from an infected human liver and used to construct subgenomic selectable replicons. Upon transfection into a human hepatoma cell line, these RNAs were found to replicate to high levels, permitting metabolic radiolabeling of viral RNA and proteins. This work defines the structure of HCV replicons functional in cell culture and provides the basis for a long-sought cellular system that should allow detailed molecular studies of HCV and the development of antiviral drugs.

13/3,AB/10 (Item 1 from file: 399)

DIALOG(R) File 399: CA SEARCH(R)

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133276316 CA: 133(20)276316e PATENT

Hepatitis C virus cell culture system for development and evaluation of antiviral agents

INVENTOR(AUTHOR): Bartenschlager, Ralf

LOCATION: Germany,

ASSIGNEE: Johannes-Gutenberg-Universitaet Mainz

PATENT: Germany Offen. ; DE 19915178 A1 DATE: 20001005

APPLICATION: DE 19915178 (19990403)

PAGES: 58 pp. CODEN: GWXXBX LANGUAGE: German CLASS: C12N-005/10A;
C12N-007/01B; A61K-048/00B

13/3,AB/11 (Item 1 from file: 340)

DIALOG(R) File 340: CLAIMS(R)/US Patent

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Dialog Acc No: 3394475 IFI Acc No: 0032384

Document Type: C

**FUNCTIONAL DNA CLONE FOR HEPATITIS C VIRUS (HCV) AND USES THEREOF;
COMPLEMENTARY NUCLEOTIDE SEQUENCES WHICH CODE REPLICATING VIRAL
TRANSCRIPTS; FOR DETECTION OF INFECTIOUS AGENT; FOR VACCINE DEVELOPMENT;
FOR DEVELOPING MODELS FOR VIRAL INFECTION OR TESTING VIRICIDES**

Inventors: Kolykhalov Alexander A (US); Rice Charles M (US)

Assignee: Washington University St Louis Assignee Code: 90682

Publication (No,Date), Applic (No,Date)

US 6127116 20001003 US 97811566 19970304

Publication Kind: A

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Cont.-in-part Pub(No), Applic(No,Date): US 5874565

US

Abstract:

The present invention relates to the determination of an authentic HCV genome RNA sequences, to construction of infectious HCV DNA clones, and to use of the clones, or their derivatives, in therapeutic, vaccine, and diagnostic applications. The invention is also directed to HCV vectors, e.g., for gene therapy of gene vaccines.

13/3,AB/12 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS
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01198522

Hepatitis C Virus cell culture system
Hepatitis C Virus Zellkultursystem
Systeme de culture du virus de l'hepatite C

PATENT ASSIGNEE:

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INVENTOR:

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PATENT (CC, No, Kind, Date): EP 1043399 A2 001011 (Basic)

APPLICATION (CC, No, Date): EP 105929 000323;

PRIORITY (CC, No, Date): DE 19915178 990403

DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI;
LU; MC; NL; PT; SE

EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI

INTERNATIONAL PATENT CLASS: C12N-015/86; C12N-007/01; C12N-007/04;

C12N-005/10; C07K-014/18; A61K-049/00; A61K-048/00

ABSTRACT EP 1043399 A2

Das erfindungsgemase Hepatitis C Virus (HCV) Zellkultursystem besteht aus humanen Hepatomazellen, die mit einem HCV-RNA-Konstrukt transfiziert sind, das die HCV-spezifischen RNA-Abschnitte 5' NTR, NS3, NS4A, NS4B, NS5A, NS5B und 3' NTR und zudem wenigstens ein selektierbares Markergen (Selektionsgen) umfasst.

ABSTRACT WORD COUNT: 40

LANGUAGE (Publication,Procedural,Application): German; German; German

FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
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SPEC A	(German)	200041	12646
Total word count - document A			13693
Total word count - document B			0
Total word count - documents A + B			13693

13/3,AB/14 (Item 2 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT
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00498491

HEPATITIS C VIRUS NS5B TRUNCATED PROTEIN AND METHODS THEREOF TO
IDENTIFY ANTIVIRAL COMPOUNDS
PROTEINE TRONQUEE DU VIRUS NS5B DE L'HEPATITE C ET PROCEDES ASSOCIES
D'IDENTIFICATION DE COMPOSES ANTIVIRAUX

Patent Applicant/Assignee:

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DEL VECCHIO Alfred,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9929843 A1 19990617

Application: WO 98US26070 19981209 (PCT/WO US9826070)

Priority Application: US 9769208 19971211

Designated States: AL AU BA BB BG BR CA CN CZ EE GE HU ID IL IS JP KP KR LC
LK LR LT LV MG MK MN MX NO NZ PL RO SG SI SK SL TR TT UA US UZ VN YU GH
GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES
FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN
TD TG

Publication Language: English

Fulltext Word Count: 12694

English Abstract

The invention provides HCV NS5B polypeptides and DNA (RNA) encoding HCV NS5B polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are methods for utilizing HCV NS5B polypeptides to screen for antiviral compounds.

French Abstract

L'invention porte sur des polypeptides du virus NS5B (HCV NS5B) de l'hépatite C et l'ADN (ARN) codant pour eux, sur des procédés d'obtention desdits polypeptides par des techniques de recombinaison, et également sur des procédés recourant aux polypeptides HCV NS5B pour le criblage de composés antiviraux.

13/3,AB/15 (Item 3 from file: 349)

DIALOG(R) File 349:PCT FULLTEXT

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00448567

**FUNCTIONAL DNA CLONE FOR HEPATITIS C VIRUS (HCV) AND USES THEREOF
CLONE D'ADN FONCTIONNEL DU VIRUS DE L'HEPATITE C (HCV) ET SES
UTILISATIONS**

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Inventor(s):

RICE Charles M III,
KOLYKHALOV Alexander A,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9839031 A1 19980911

Application: WO 98US4428 19980226 (PCT/WO US9804428)

Priority Application: US 97811566 19970304

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES
FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD
MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US
UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE
CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML
MR NE SN TD TG

Publication Language: English

Fulltext Word Count: 51598

English Abstract

The present invention relates to the determination of an authentic HCV genome RNA sequences, to construction of infectious HCV DNA clones, and to use of the clones, or their derivatives, in therapeutic, vaccine, and diagnostic applications. The invention is also directed to HCV vectors, e. g., for gene therapy of gene vaccines.

French Abstract

La présente invention concerne l'analyse de séquences d'ARN génomique d'un authentique HCV, la construction de clones d'ADN du HCV infectieux, et l'utilisation de ces clones, ou de leurs dérivés, dans des applications thérapeutique, vaccinale et diagnostique. Cette invention concerne également des vecteurs HCV, utilisés par exemple en thérapie génique ou pour les vaccins géniques.

13/3,AB/17 (Item 1 from file: 654)
DIALOG(R) File 654:US PAT.FULL.
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03194973

Utility

FUNCTIONAL DNA CLONE FOR **HEPATITIS C VIRUS (HCV)** AND USES THEREOF

PATENT NO.: 6,127,116
ISSUED: October 03, 2000 (20001003)
INVENTOR(s): Rice, Charles M., University City, MO (Missouri), US (United States of America)
Kolykhalov, Alexander A., St. Louis, MO (Missouri), US (United States of America)
ASSIGNEE(s): Washington University, (A U.S. Company or Corporation), St. Louis, MO (Missouri), US (United States of America)
[Assignee Code(s): 90682]
APPL. NO.: 8-811,566
FILED: March 04, 1997 (19970304)

RELATED APPLICATIONS

The application is a continuation-in-part of and claims priority to U.S. Ser. No. 08-520,678 filed Aug. 29, 1995, now U.S. Pat. No. 5,874,565, which issued Feb. 23, 1999.

GOVERNMENT SUPPORT

The research leading to the present invention was supported, at least in part, by grants from United States Public Health Service Grant Nos. CA57973 and A131501. Accordingly, the Government may have certain rights in the invention.

FULL TEXT: 5799 lines

ABSTRACT

The present invention relates to the determination of an authentic HCV genome RNA sequences, to construction of infectious HCV DNA clones, and to use of the clones, or their derivatives, in therapeutic, vaccine, and diagnostic applications. The invention is also directed to HCV vectors, e.g., for gene therapy of gene vaccines.

13/3,AB/18 (Item 2 from file: 654)
DIALOG(R) File 654:US PAT.FULL.
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02916221

Utility

NUCLEIC ACIDS COMPRISING A HIGHLY CONSERVED NOVEL 3 TERMINAL SEQUENCE ELEMENT OF THE **HEPATITIS C VIRUS**
[Genetic engineered DNA or RNA molecule having a hepatitis C virus terminal nucleic acid sequence use for nucleic-acid based diagnostics and for developing anti-hepatitis C virus therapies, vaccines against hepatitis]

PATENT NO.: 5,874,565
ISSUED: February 23, 1999 (19990223)
INVENTOR(s): Rice, Charles M., University City, MO (Missouri), US (United States of America)
Kolykhalov, Alexander A., St. Louis, MO (Missouri), US (United States of America)
ASSIGNEE(s): Washington University, (A U.S. Company or Corporation), St. Louis, MO (Missouri), US (United States of America)
[Assignee Code(s): 90682]

28/3,AB/13 (Item 3 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE
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01624385 2000284235

Efficient initiation of HCV RNA replication in cell culture

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Journal: Science, 290/5498 (1972-1974), 2000, United States

PUBLICATION DATE: December 8, 2000

CODEN: SCIEA

ISSN: 0036-8075

DOCUMENT TYPE: Article

LANGUAGES: English SUMMARY LANGUAGES: English

NO. OF REFERENCES: 31

Hepatitis C virus (HCV) infection is a global health problem affecting an estimated 170 million individuals worldwide. We report the identification of multiple independent adaptive mutations that cluster in the HCV nonstructural protein **NS5A** and confer increased replicative ability in vitro. Among these adaptive mutations were a single amino acid substitution that allowed HCV RNA replication in 10% of transfected hepatoma cells and a deletion of 47 amino acids encompassing the interferon (IFN) sensitivity determining region (ISDR). Independent of the ISDR, IFN-alpha rapidly inhibited HCV RNA replication in vitro. This work establishes a robust, cell-based system for genetic and functional analyses of HCV replication.

28/3,AB/19 (Item 9 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE
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01555351 1999084877

Characterization of the effects of hepatitis C virus nonstructural 5A protein expression in human cell lines and on interferon-sensitive virus replication

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Journal: Hepatology, 29/4 (1262-1271), 1999, United States

CODEN: HPTLD

ISSN: 0270-9139

DOCUMENT TYPE: Article

LANGUAGES: English SUMMARY LANGUAGES: English

NO. OF REFERENCES: 62

The hepatitis C virus (HCV) nonstructural 5A (**NS5A**) protein has been implicated in the inherent resistance of HCV to interferon (IFN) antiviral therapy in clinical studies. Biochemical studies have demonstrated that **NS5A** interacts in vitro with and inhibits the IFN-induced, RNA-dependent protein kinase, PKR, and that **NS5A** interacts with at least one other cellular kinase. The present study describes the establishment and characterization of various stable **NS5A** -expressing human cell lines, and the development of a cell culture-based assay for determining the inherent IFN resistance of clinical **NS5A** isolates. Human epithelioid (Hela) and osteosarcoma (U2-OS) cell lines were generated that express **NS5A** under tight regulation by the tetracycline- dependent promoter. Maximal expression of **NS5A** occurred at 48 hours following the removal of tetracycline from the culture medium. The half-life of **NS5A** in these cell lines was between 4 to 6 hours. **NS5A** protein expression was localized cytoplasmically, with a staining pattern consistent with the location of the Golgi apparatus and endoplasmic reticulum. In the majority of cell lines, no Obvious phenotypic changes were observed. However, three genotype 1b **NS5A** -expressing osteosarcoma cell lines exhibited cytopathic effect

and severely reduced proliferation as a result of high-level **NS5A** expression. Full-length **NS5A** protein isolated from a genotype 1b IFN-nonresponsive patient (**NS5A** -1b) was capable of rescuing encephalomyocarditis virus replication during IFN challenge up to 40-fold, whereas a full-length **NS5A** -1a and an interferon sensitivity determining region (ISDR) deletion mutant (**NS5A** -1a-DeltaISDR) isolated from a genotype 1a IFN-nonresponsive patient showed no rescue activity. The **NS5A** -1b and **NS5A** -1a proteins also rescued vesicular stomatitis virus replication during IFN treatment by two- to threefold. These data cumulatively suggest that **NS5A** expression alone can render cells partially resistant to the effects of IFN against IFN-sensitive viruses, and that in some systems, these effects may be independent of the putative ISDR. A scenario is discussed in which the **NS5A** protein may employ multiple strategies contributing to IFN resistance during HCV infection.

28/3,AB/41 (Item 31 from file: 71)
 DIALOG(R)File 71:ELSEVIER BIOBASE
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01136852 1999106015
 Hepatitis C virus **NS5A** protein modulates cell cycle regulatory genes and promotes cell growth
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 Journal: Journal of General Virology, 80/5 (1179-1183), 1999, United Kingdom
 CODEN: JGVIA
 ISSN: 0022-1317
 DOCUMENT TYPE: Article
 LANGUAGES: English SUMMARY LANGUAGES: English
 NO. OF REFERENCES: 36

The phosphoprotein **NS5A** of hepatitis C virus has recently been suggested to control PKR protein kinase for resistance to interferon. To investigate other functions of **NS5A**, studies were initiated on the regulation of transcription of important cellular genes and of cell growth by this protein. The results suggested that **NS5A** protein represses transcription of the cell cycle regulatory gene p21(WAF1), while it activates the human proliferating cell nuclear antigen gene in murine fibroblasts and human hepatoma cells. Furthermore, introduction of **NS5A** into murine fibroblasts (NIH3T3) promoted anchorage-independent growth and tumour formation in nude mice. Thus, **NS5A** appears to exhibit a role in cell growth regulation.

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 DIALOG(R)File 71:ELSEVIER BIOBASE
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00326822 96017878
 Virion-like structures in HeLa G cells transfected with the full-length sequence of the hepatitis C virus genome
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 ADDRESS: Dr. M. Mizuno, First Dept. of Internal Medicine, Okayama University Medical School, 2-5-1, Shikata-cho, Okayama 700, Japan
 Journal: Gastroenterology, 109/6 (1933-1940), 1995, United States
 PUBLICATION DATE: 19950000
 CODEN: GASTA
 ISSN: 0016-5085
 DOCUMENT TYPE: Article
 LANGUAGES: English SUMMARY LANGUAGES: English

Background and Aims: The process and the site of hepatitis C virus (HCV) particle formation in cells after infection remain unknown. The aim of this study was to create an in vitro model for the study of HCV particle formation. Methods: HeLa G cells were transfected with the full-length sequence of the HCV genome. Viral protein expression was analyzed using

immunoblotting. The cells were examined using immunoelectron and conventional electron microscopy. Results: Core, E2, NS3, **NS5a**, and NSSb proteins were identified using immunoblotting. Immunoelectron microscopy showed that the core antigen was located along the membrane of the endoplasmic reticulum (ER) and occasionally in its cisternae, Core antigen-positive particles of 30 nm in diameter were found in the cytosol and in the cisternae of the ER. The particles in the cisternae were coated with an outer membrane that was connected to the ER membrane. Conventional electron microscopy revealed particles of 45 nm in diameter with electrondense cores in the cisternae of the ER. The outer membrane of the particles was occasionally connected to the ER membrane. Conclusions: The findings suggest that HCV core proteins are synthesized and assembled into particles in the cytosol and that they bud into the cisternae of the ER to form coated particles.

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28/3,AB/2 (Item 2 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

10776143 20190256 PMID: 10726057

Molecular virology of hepatitis C virus : an update with respect to potential antiviral targets.

Blight KJ; Kolykhalov AA; Reed KE; Agapov EV; Rice CM
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Antiviral therapy (ENGLAND) 1998, 3 (Suppl 3) p71-81, ISSN 1359-6535 Journal Code: DIV

Contract/Grant No.: AI40034, AI, NIAID; CA57973, CA, NCI

Languages: ENGLISH

Document type: Journal Article; Review; Review, Tutorial

Record type: Completed

Hepatitis C virus (HCV), a positive-strand enveloped RNA virus, is a major cause of chronic liver disease worldwide. Cis-acting RNA elements and virus-encoded polypeptides required for HCV replication represent attractive targets for the development of antiviral therapies. Internal ribosome entry site-directed translation of HCV genome RNA produces a long polyprotein which is co- and post-translationally processed to yield at least 10 viral proteins. A host signal peptidase is responsible for maturation of the structural proteins located in the N-terminal one-third of the polyprotein. Thus far, four enzymatic activities encoded by the non-structural (NS) proteins have been reported. The NS2-3 region encodes an autoprotease responsible for cleavage at the 2/3 site. The N-terminal one-third of NS3 functions as the catalytic subunit of a serine proteinase which cleaves at the 3/4A, 4A/4B, 4B/5A and 5A/5B sites, and NS4A is an essential cofactor for some of these cleavages. NS3 also encodes an RNA-stimulated NTPase/RNA helicase at its C terminus, and NS5B has been shown to possess an RNA-dependent RNA polymerase activity. To date, no functions have been reported for NS4B or NS5A in RNA replication, however, NS5A has been implicated in modulating the sensitivity of HCV to interferon. Sequence and structural conservation within the 3' terminal 98 bases of genomic RNA suggest a functional importance in the virus life-cycle and hence another target for antiviral intervention. Recently, HCV infection was shown to be initiated in chimpanzees following intrahepatic inoculation of RNA transcribed from cloned HCV cDNA. The ability to generate large quantities of infectious HCV RNA may facilitate the development of reliable cell culture replication systems useful for the evaluation of antiviral drugs.

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DIALOG(R) File 34:SciSearch(R) Cited Ref Sci
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07263494 Genuine Article#: 143QP Number of References: 152

Title: Molecular virology of hepatitis C virus : an update with respect to potential antiviral targets (ABSTRACT AVAILABLE)

Author(s): Blight KJ; Kolykhalov AA; Reed KE; Vagapov EV; Rice CM
(REPRINT)

Corporate Source: WASHINGTON UNIV,SCH MED, DEPT MOL MICROBIOL, BOX 8230,
660 S EUCLID AVE/ST LOUIS//MO/63110 (REPRINT); WASHINGTON UNIV,SCH MED,
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Journal: ANTIVIRAL THERAPY, 1998, V3, 3, P71-81

ISSN: 1359-6535 Publication date: 19980000

Publisher: INT MEDICAL PRESS, 125 HIGH HOLBORN, LONDON WC1V 6QA, ENGLAND

Language: English Document Type: ARTICLE

Abstract: Hepatitis C virus (HCV), a positive-strand enveloped RNA virus, is a major cause of chronic liver disease worldwide. Cis-acting RNA elements and virus-encoded polypeptides required for HCV replication represent attractive targets for the development of antiviral therapies. Internal ribosome entry site-directed translation of HCV genome RNA produces a long polyprotein which is co- and post-translationally processed to yield at least 10 viral proteins. A host signal peptidase is responsible for maturation of the structural proteins located in the N-terminal one-third of the polyprotein. Thus

far, four enzymatic activities encoded by the non-structural (NS) proteins have been reported. The NS2-3 region encodes an autoprotease responsible for cleavage at the 2/3 site. The N-terminal one-third of NS3 functions as the catalytic subunit of a serine proteinase which cleaves at the 3/4A, 4A/4B, 4B/5A and 5A/5B sites, and NS4A is an essential cofactor for some of these cleavages. NS3 also encodes an RNA-stimulated NTPase/RNA helicase at its C terminus, and NS5B has been shown to possess an RNA-dependent RNA polymerase activity. To date, no functions have been reported for NS4B or **NS5A** in RNA replication, however, **NS5A** has been implicated in modulating the sensitivity of HCV to interferon. Sequence and structural conservation within the 3' terminal 98 bases of genomic RNA suggest a functional importance in the virus life-cycle and hence another target for antiviral intervention. Recently, HCV infection was shown to be initiated in chimpanzees following intrahepatic inoculation of RNA transcribed from cloned HCV cDNA. The ability to generate large quantities of **infectious** HCV RNA may facilitate the development of reliable cell culture replication systems useful for the evaluation of antiviral drugs.

28/3,AB/16 (Item 6 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE
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01574918 2000233977

Integrity of the NS5A (amino acid 2209 to 2248) region in hepatitis C virus 1b patients non-responsive to interferon therapy

Halfon P.; Halimi G.; Bourliere M.; Ouzan D.; Durant J.; Khiri H.; Mercier L.; Gerolami V.; Cartouzou G.

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Journal: Liver, 20/5 (381-386), 2000, Denmark

CODEN: LIVED

ISSN: 0106-9543

DOCUMENT TYPE: Article

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NO. OF REFERENCES: 36

Background/Aims: In hepatitis C virus-1b, it has been suggested that an amino acid stretch (aa 2209-2248) of the carboxy terminal half of the non-structural 5A (**NS5A**) region participates in the response to interferon treatment. We tested the hypothesis that absence of mutations in the **NS5A** (aa 2209-2248) sequence is required for interferon resistance. We also investigated the importance of different HCV-1b isolates in interferon response in France. Methods: We determined the **NS5A** sequences of 70 patients with chronic hepatitis C before IFN therapy and then compared them with HCV-J prototype sequence. The isolates were determined by NS5B sequencing, the "gold standard" method for genotyping and subtyping. Pre-therapeutic viral load was also measured. Results: No sustained virological response was observed in the patients without amino acid substitutions in the **NS5A** (aa 2209-2248) sequence, and in the patients with HCV-J isolates. Viral load was significantly higher in the patients with no amino acid substitutions in the **NS5A** (aa 2209-2248) sequence. Conclusions: In HCV-1b infected patients, an HCV-J strain with no amino acid substitution in the **NS5A** (aa 2209-2248) region indicates a poor prognosis for response to IFN therapy. The low interferon response rate in HCV-1b infection in Europe is probably not due to a difference between isolates.

28/3,AB/18 (Item 8 from file: 71)
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01561116 2000220841

Nuclear localization and intramolecular cleavage of N-terminally deleted NS5A protein of hepatitis C virus

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Journal: Virus Research, 69/2 (109-117), 2000, Netherlands

PUBLICATION DATE: September 25, 2000

CODEN: VIRED

ISSN: 0168-1702

PUBLISHER ITEM IDENTIFIER: S0168170200002069

DOCUMENT TYPE: Article

LANGUAGES: English SUMMARY LANGUAGES: English

NO. OF REFERENCES: 34

The full-size **NS5A** (**NS5A** -F) of hepatitis C virus is localized in the cytoplasm despite the presence of a functional nuclear localization signal (NLS) in its C-terminal region (amino acids (aa) 354-362). In the present study, we demonstrated that a short stretch of sequence near the N-terminus of **NS5A** (aa 27-38) masked the functional NLS, preventing **NS5A** from being transported to the nucleus. This sequence, referred to as an NLS-masking sequence, was distinct from a nuclear export signal, as it did not actively target a protein to the cytoplasm. We also found that other sequences located at either an N- (aa 1-21) or a C-terminal region (aa 353-447) were responsible for targeting **NS5A** to the cytoplasm. Western blot analysis of the transfected cells revealed that **NS5A** mutants that had been N-terminally deleted by 66 aa or more were cleaved at a certain cleavage site, generating a common fragment of ca. 40 kDa. This result implies the possible presence of a cleavage site in the **NS5A** sequence around aa 150, which is exposed through conformational alteration upon the N-terminal deletions. (C) 2000 Elsevier Science B.V.

28/3,AB/22 (Item 12 from file: 71)

DIALOG(R)File 71:ELSEVIER BIOBASE

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01456633

2000132464

Mutations within the E2 and NS5A protein in patients infected with

hepatitis C virus type 3a and correlation with treatment response
Sarrazin C.; Kornetzky I.; Ruster B.; Lee J.-H.; Kronenberger B.; Bruch K.;
Roth W.K.; Zeuzem S.

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Defined regions of hepatitis C virus (HCV) envelope 2 (E2), PePHD, and nonstructural 5A (**NS5A**) protein (PKR-binding domain) have been shown to interact with interferon alpha (IFN-alpha)-inducible double-stranded RNA-activated protein kinase (PKR) in vitro, suggesting a possible mechanism of HCV to evade antiviral effects of IFN-alpha. The clinical correlation between amino acid mutations within the E2 PePHD or the **NS5A** PKR-binding domain and response to antiviral treatment in HCV-3a-infected patients is unknown. Thirty-three patients infected with HCV-3a isolates were treated with IFN-alpha with or without ribavirin. The carboxyterminal half of E2 and of the **NS5A** gene were sequenced. Sixteen patients achieved a sustained virological response (SR), 6 patients an end-of-treatment response with relapse thereafter (ETR), and 11 patients were nonresponders (NR). Within the PePHD of the E2 protein 0.5 (range, 0-2) mutations were observed in SR patients, whereas the number of mutations in ETR or NR patients was 0.2 (0-1). Quasispecies analyses showed almost no heterogeneity. The mean number of mutations within the PKR-binding domain of the **NS5A** protein was 1.6 (range, 0-4) in SR patients, 1 (0-2) in ETR patients, and 1.6 (0-3) in NR patients. Patients with higher numbers of

mutations within the E2 or **NS5A** region showed a trend towards lower pretreatment viremia. Phylogenetic and conformational analyses of E2 or **NS5A** sequences allowed no differentiation between sensitive and resistant isolates. However, mutations within the E2 PePHD in SR patients were frequent, and hydrophobic mutations within the hydrophilic area of PePHD at codon 668 and 669 were exclusively observed in sustained virological responders.

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Nonstructural protein 5A of hepatitis C virus inhibits the function of karyopherin beta3

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It has been suggested that nonstructural protein 5A (**NS5A**) of hepatitis C virus (HCV) plays a role in the incapacitation of interferon by inactivation of RNA-dependent protein kinase PKR. In order to further investigate the role of **NS5A** , we tried to identify cellular proteins interacting with **NS5A** by using the yeast two-hybrid system. The karyopherin beta3 gene was isolated from a human liver cell library as a protein interacting with **NS5A** . The protein-protein interaction between **NS5A** and karyopherin beta3 was confirmed by in vitro binding assay and an in vivo coimmunoprecipitation method. The effect of **NS5A** on the karyopherin beta3 activity was investigated using a yeast cell line containing mutations in both PSE1 and KAP123, genes that are homologous to the human karyopherin beta3 gene. Human karyopherin beta3 complemented the loss of the PSE1 and KAP123 functions, supporting growth of the double mutant cells. However, expression of **NS5A** hampered the growth of the double mutant cells supplemented with human karyopherin beta3. On the other hand, expression of **NS5A** by itself had no effect on the growth of the double mutant expressing wild-type yeast PSE1. This indicates that **NS5A** may inhibit karyopherin beta3 function via protein- protein interaction. The role of **NS5A** in HCV replication is discussed.

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Mutations in the protein kinase-binding domain of the NS5A protein in patients infected with hepatitis C virus type 1a are associated with treatment response

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An interaction of the hepatitis C virus (HCV) **NS5A** protein with the interferon (IFN)-alpha-inducible double-stranded RNA-activated protein kinase (PKR) was demonstrated in vitro. The clinical correlation between amino acid mutations within the HCV **NS5A** region and response to antiviral treatment is controversial. Thirty-two patients chronically infected with HCV-1a, who were treated with IFN-alpha with or without ribavirin, were studied. The carboxy-terminal half of HCV **NS5A** was sequenced and was investigated by phylogenetic and conformational analyses. Eight patients achieved a sustained virologic response. An end-of-treatment response but relapse thereafter was observed among 8 patients, whereas 16 patients were nonresponders. The median number of mutations within the PKR-binding domain but not within the previously described IFN sensitivity-determining region was significantly higher for patients with sustained (3 mutations [range, 1- 5]) or end-of-treatment (4 mutations [range, 1-5]) virologic response than for nonresponders (2 mutations [range, 0-3]) (P = .0087). Phylogenetic and conformational analyses of **NS5A** sequences allowed no differentiation between sensitive and resistant strains.

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Specific interaction between the hepatitis C virus NS5B RNA polymerase and the 3' end of the viral RNA

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Hepatitis C virus (HCV) NS5B protein is the viral RNA-dependent RNA polymerase capable of directing RNA synthesis. In this study, an electrophoretic mobility shift assay demonstrated the interaction between a partially purified recombinant NS5B protein and a 3' viral genomic RNA with or without the conserved 98-nucleotide tail. The NS5B-RNA complexes were specifically competed away by the unlabeled homologous RNA but not by the viral 5' noncoding region and very poorly by the 3' conserved 98-nucleotide tail. A 3' coding region with conserved stem-loop structures rather than the 3' noncoding region of the HCV genome is critical for the specific binding of NS5B. Nevertheless, no direct interaction between the 3' coding region and the HCV **NS5A** protein was detected. Furthermore, two independent RNA-binding domains (RBDs) of NS5B were identified, RBD1, from amino acid residues 83 to 194, and RBD2, from residues 196 to 298. Interestingly, the conserved motifs of RNA-dependent RNA polymerase for putative RNA binding (220-DxxxxD-225) and template/primer position (282-S/TGxxxTxxxNS/T-292) are present in the RBD2. Nevertheless, the RNA-binding activity of RBD2 was abolished when it was linked to the carboxy-terminal half of the NS5B. These results provide some clues to understanding the initiation of HCV replication.

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Continuous human cell lines inducibly expressing hepatitis C virus structural and nonstructural proteins

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Investigation of the hepatitis C virus (HCV) life cycle and the evaluation of novel antiviral strategies are limited by the lack of an efficient cell culture system. Therefore, continuous human cell lines inducibly expressing the entire HCV open reading frame were generated with use of a tetracycline-regulated gene expression system. HCV transgenes were found to be chromosomally integrated in a head-to-tail configuration. Northern blot analyses revealed a tightly regulated unspliced transcript of approximately 9 kilobases (kb). HCV structural and nonstructural proteins were faithfully processed, indicating that the cellular and viral proteolytic machineries and posttranslational modification pathways are fully functional in these cell lines. Steady state expression levels could be regulated over a broad range by the concentration of tetracycline present in the culture medium. Kinetic analyses revealed a half-life of less than 1 hour for the HCV RNA whereas a half-life of approximately 9.5, 12, 11, and 10 hours was found for core, NS3, NS4A, and NS5A proteins, respectively. Viral proteins were found to colocalize in the cytoplasm in a pattern characteristic of the endoplasmic reticulum. High-level expression of HCV proteins in the fully induced state was toxic to the cells. These cell lines provide a unique in vitro system to analyze structural and functional properties of HCV proteins, their interactions with cellular proteins and pathways, and the requirements for HCV morphogenesis. In addition, they should prove useful for the evaluation of novel antiviral strategies against hepatitis C in a well-defined and reproducible cellular context.

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The nonstructural proteins of the hepatitis C virus : Structure and functions
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The hepatitis C virus is the major causative agent of nonA-nonB hepatitis worldwide. Although this virus cannot be cultivated in cell culture, several of its features have been elucidated in the past few years. The viral genome is a single-stranded, 9.5 kb long RNA molecule of positive polarity. The viral genome is translated into a single polyprotein of about 3000 amino acids. The virally encoded polyprotein undergoes proteolytic processing by a combination of cellular and viral proteolytic enzymes in order to yield all the mature viral gene products. The gene order of HCV has been determined to be C-E1-E2-p7-NS2-NS3-NS4A-NS4B- NS5A -NS5B. The mature structural proteins, C, E1 and E2 have been shown to arise from the viral polyprotein via proteolytic processing by host signal peptidases. Conversely, generation of the mature nonstructural proteins relies on the activity of viral proteases. Thus, cleavage at the NS2/NS3 junction is accomplished by a metal-dependent autoprotease encoded within NS2 and the N-terminus of NS3. The remaining cleavages downstream from this site are

effected by a serine protease contained within the N-terminal region of NS3. Besides the protease domain, NS3 also contains an RNA helicase domain at its C-terminus, NS3 forms a heterodimeric complex with NS4A. The latter is a membrane protein that has been shown to act as a cofactor of the protease. Whereas the NS5B protein has been shown to be the viral RNA-dependent RNA polymerase, no function has yet been attributed to NS4B and **NS5A**. The latter is a cytoplasmic phosphoprotein and appears to be involved in mediating the resistance of the hepatitis C virus to the action of interferon.

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Molecular virology of hepatitis C virus : An update with respect to potential antiviral targets
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Hepatitis C virus (HCV), a positive-strand enveloped RNA virus, is a major cause of chronic liver disease worldwide. Cis-acting RNA elements and virus-encoded polypeptides required for HCV replication represent attractive targets for the development of antiviral therapies. Internal ribosome entry site-directed translation of HCV genome RNA produces a long polyprotein which is co- and post-translationally processed to yield at least 10 viral proteins. A host signal peptidase is responsible for maturation of the structural proteins located in the N-terminal one-third of the polyprotein. Thus far, four enzymatic activities encoded by the non-structural (NS) proteins have been reported. The NS2-3 region encodes an autoprotease responsible for cleavage at the 2/3 site. The N-terminal one-third of NS3 functions as the catalytic subunit of a serine proteinase which cleaves at the 3/4A, 4A/4B, 4B/5A and 5A/5B sites, and NS4A is an essential cofactor for some of these cleavages. NS3 also encodes an RNA-stimulated NTPase/RNA helicase at its C terminus, and NS5B has been shown to possess an RNA-dependent RNA polymerase activity. To date, no functions have been reported for NS4B or **NS5A** in RNA replication, however, **NS5A** has been implicated in modulating the sensitivity of HCV to interferon. Sequence and structural conservation within the 3' terminal 98 bases of genomic RNA suggest a functional importance in the virus life-cycle and hence another target for antiviral intervention. Recently, HCV infection was shown to be initiated in chimpanzees following intrahepatic inoculation of RNA transcribed from cloned HCV cDNA. The ability to generate large quantities of **infectious** HCV RNA may facilitate the development of reliable cell culture replication systems useful for the evaluation of antiviral drugs.

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